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TITLE A MODEL FOR ANTIBODY MEDIATED CELL AGGREGATION: ROSETTE FORMATION

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A MODEL FOR ANTIBODY MEDIATED CELL AGGREGATION: ROSETTE FORMATION

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Models are developed for the formation of cellular aggregates, called rosettes, composed of a central lymphocyte and surrounding antibody coated red blood cells. Kinetic and equilibrium models are considered from both the deterministic and stochastic viewpoints. Analytic solutions are given to the system of nonlinear ordinary differential equations that describe the formation of different size rosettes in the cases of both reversible and irreversible red cell adhesion. A comparison of the stochastic model with experimental data indicates that there may exist heterogeneity within the lymphocyte population with regard to the number of chemical bonds required to bind a red cell to a lymphocyte.

1. INTRODUCTION

The adhesion of cells mediated by both specific and nonspecific interactions is of fundamental importance in determining the morphology and physiology of both cells and multicellular organisms.[1] As Warren Lewis put it in 1923: "Were the various types of cells to lose their stickiness for one another and for the supporting extracellular white fibers, reticuli, etc., our bodies would at once disintegrate and flow off into the ground in a mixed stream of ectodermal, muscle, mesenchyme, endothelial, liver, pancreatic, and many other types of cells."[2] Despite the fundamental importance of cell-cell adhesion, it has only been in recent years that rigorous quantitative models of cell-cell adhesion have been developed.[3-8] Current models tend to analyze in great detail the interaction between pairs of cells. It would be of value to build upon the knowledge of pairwise interactions and develop models for the formation of multicellular aggregates. In this paper I will carry out this program for a very specific case of interest in immunology -- rosette formation. A rosette consists of a central cell surrounded by a cluster of adhering cells (see Fig. 1).

Rosette formation is used in immunology as an assay technique for detecting the presence of cell surface receptors. In the system to be modeled, the central cell is a lymphocyte and the surrounding cells are antibody coated red blood cells (RBC). As shown in Fig. 1 when antibody binds to a RBC the Fc portion of the antibody or immunoglobulin (Ig) molecule remains free. A cell such as a lymphocyte or a macrophage that has on its surface Fc receptors, i.e., proteins that can bind the Fc portion of Ig molecules, is capable of having antibody coated RBC adhere to it. Adhesion between the RBC and the lymphocyte is mediated by the specific interaction of Fc regions on the antibody

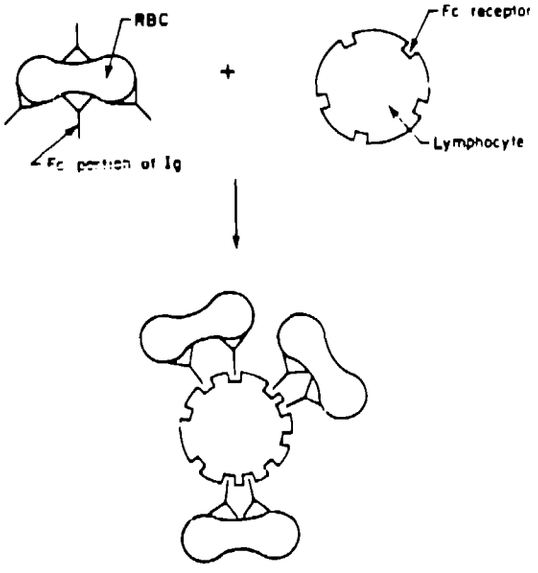

Figure 1: A schematic illustration of a rosette forming experiment. Red blood cells (RBC) are incubated in the presence of antibody or immunoglobulin (Ig) molecules and then washed. The Ig, which are depicted as Y shaped molecules, bind to the RBC, with the tail of the Y, the Fc portion of the Ig molecule, pointing into the solution. A lymphocyte has on its surface Fc receptors that specifically bind the Fc portion of Ig. When antibody coated RBC are mixed with lymphocytes, Fc receptor-Ig bonds are formed that mediate the adhesion of a red cell to a lymphocyte. The number of chemical bonds, N, required to attach the red cell to the lymphocyte may vary with the affinity of the Fc receptor for Ig. In the figure N=2.

coated red cells and the Fc receptor on the lymphocyte. A rosette assay of this type is called an EA-rosette assay because it employs erythrocytes (red cells) coated with antibody. Experimentally, EA-rosette assays are used to detect either the presence of Fc receptors on cells or the presence of specific molecules on the surface of RBC which the antibody that coats the RBC recognizes.

2. ASSUMPTIONS UNDERLYING THE MODEL

We consider rosettes formed by antibody coated RBC binding to lymphocytes. We assume:

a) Red cells are indistinguishable, and each has γ randomly distributed Ig molecules irreversibly attached to its surface. γ is a parameter of the model, an increasing function of the Ig concentration used in preparing the antibody coated RBC.

b) The lymphocytes that form rosettes may be heterogeneous with respect to the number of Fc receptors per cell. On each cell the equilibrium binding constant (affinity) of the Fc receptors for Ig is assumed to be a constant, but this affinity may vary from cell to cell.

c) In order for a RBC to adhere to a lymphocyte there must be at least N Ig molecules in an area δA confronting the lymphocyte. N presumably will vary with the number and affinity of Fc receptors on the lymphocyte. δA may be the whole contact area between the lymphocyte and the RBC, or it might be a smaller area, such as the ends of microvilli, in which the Ig Fc receptor interactions are concentrated.

d) There is a maximum number, M, of RBC which can crowd around a lymphocyte to form a rosette.

e) Attachment of successive RBC to a lymphocyte occurs independently of each other, except insofar as bound RBCs block some of the lymphocyte surface from further binding.

Based on the above assumptions we develop both deterministic and stochastic models to predict the distribution of the number of RBC attached per lymphocyte.

3. A STOCHASTIC MODEL

In the area δA the average number of Ig molecules is $\lambda = \gamma(\delta A)/A$, where A is the area of the entire red cell. Because Ig molecules are assumed to be randomly distributed on the RBC surface, the probabiltiy of finding n molecules in the area δA is given by the Poisson distribution

$$P(n;\lambda) = \frac{\lambda^n}{n!} e^{-\lambda} \quad . \tag{1}$$

According to assumption (c) a red cell will stick to a given lymphocyte if there are at least N Ig molecules in the area δA. Thus, $p(N,\lambda)$, the probability that a red cell with an average of λ Ig molecules in an area δA will stick to a lymphocyte, is given by

$$p(N,\lambda) = \sum_{n=N}^{\infty} P(n;\lambda) = 1 - \sum_{n=0}^{n-1} P(n;\lambda) \quad . \tag{2}$$

Many RBC, up to a maximum of M, can stick to a given lymphocyte. If we assume that each RBC occupies a site and that there are M sites on a lymphocyte, then the probability that exactly m RBC surround the lymphocyte is given by the binomial distribution

$$p_m(N) = \binom{M}{m} p(N,\lambda)^m [1-p(N,\lambda)]^{M-m} \tag{3}$$

The use of the binomial distribution here is clearly an approximation. A better model would allow the red cells to sterically block each other as in the classic car parking problem [9-10]. Refinements of Eq. (3) will be discussed elsewhere.

In experiments, a rosette forming cell is commonly defined as a lymphocyte surrounded by three or more RBC. Adopting this definition in order to compare theory with experiment, we find the probability that a given lymphocyte forms a rosette is

$$P_{ros}(N) = \sum_{m=3}^{M} p_m(N) = 1 - \sum_{m=0}^{2} p_m(N) \quad . \tag{4}$$

Equation (4) provides the probability of finding a rosette around a lymphocyte which requires N Ig molecules to be in the area δA confronting a RBC. Other lymphocytes in the population may require more than or fewer than N Ig in the area δA to allow sticking, because of a different number or a different affinity of their Fc receptors. If the critical number N is distributed randomly throughout the lymphocyte population with distribution f(N), i.e., f(N) is the fraction of lymphocytes which require N IgG molecules to mediate adhesion, then the probability of finding any lymphocyte surrounded by exactly m RBC is

$$p_m = \sum_N p_m(N) f(N) \quad , \tag{5}$$

and the probability that a lymphocyte is a rosette forming cell is

$$P_{ros} = \sum_{m=3}^{M} p_m = \sum_N P_{ros}(N) f(N) \quad . \tag{6}$$

In Fig. 2 we compare this model to data of Beckett, Bankhurst and Williams.[11] We find that by choosing f(N) as a truncated version of the Poisson distribution with mean μ, we get reasonable agreement to the data. We chose a truncated Poisson distribution for three reasons. First, we believed N would be distributed randomly. Second, N is an integer.

Third, the probability that two cells would stick with no bonds, or perhaps even a few bonds between them, is zero. Thus we assume

$$f(N) = 0 \quad \text{if} \quad N < N_{min} ,$$

$$f(N) = \frac{\mu^{N} e^{-\mu}}{N! \left[1 - \sum_{q=0}^{N_{min}-1} \frac{\mu^{q} e^{-\mu}}{q!} \right]}$$

$$\text{if} \quad N \geq N_{min} . \qquad (7)$$

To fit the data in Fig. 2 we chose M = 30, implying that a maximum of 30 red cells can surround a lymphocyte, which is in qualitative agreement with observations. Further in Fig. 2, $N_{min} = 2$ and $\mu = 4$, implying that at least two Fc receptor Ig bonds are required to attach a RBC to a lymphocyte, but that on average four such bonds are required. Bell [14] estimates that approximately four bonds, each with the strength of a typical antigen-antibody bond, will hold a lymphocyte to an endothelial cell in a venule subject to a mild fluid flow with a velocity of 0.3 cm/sec. Thus our estimate of μ from the data in Fig. 2 is within reason. Lastly, the implication of our data fit is that lymphocytes are heterogeneous with respect to their ability to form rosettes with RBC.

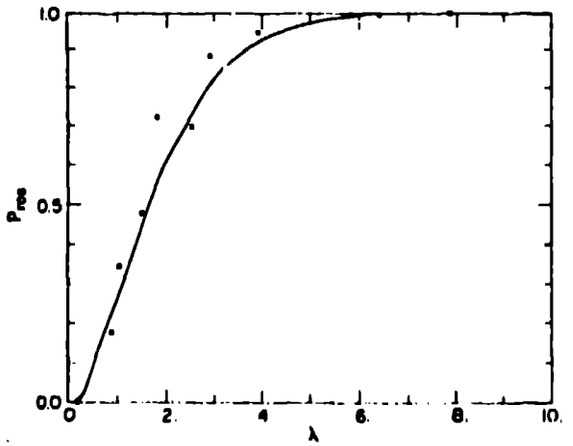

Figure 2: Comparison of the stochastic theory, as given by Eqs. (6) and (7), with data given in Fig. 2 of Beckett, Bankhurst and Williams. [11] A series of experiments were done. In each, RBC were incubated with a different dilution of Ig, and hence a different mean concentration λ of Ig can be assumed to be in the area δA. Because Beckett et al. did not determine the absolute amount of Ig on each cell an arbitrary scale factor is included in λ. In each experiment the percentage of lymphocytes that formed rosettes, i.e., had 3 or more RBC attached, was recorded. The experimental curve asymptoted at less then 100% rosette forming cells, even at the highest concentrations of Ig used, indicating that not all cells counted as lymphocytes were capable of forming rosettes. Because our theory assumes all cells are potential rosette forming cells, the data was scaled so that it asymptoted at 100%. The theoretical curve was generated from Eqs. (6) and (7) with M = 30, $N_{min} = 2$ and $\mu = 4$.

Our work in fitting the model to data is only preliminary. Choices for f(N) other than the truncated Poisson may also fit the data. Furtner, analysis of this and other data and the design of experiments that can more fully test the theory will be discussed elsewhere.

4. A KINETIC MODEL OF CELL AGGREGATION

In the case that f(N) approaches a delta function and all lymphocytes can be treated as being identical, kinetic models based on the law of mass-action can easily be constructed. Consider a system in which lymphocytes and RBC collide at random, RBC reversibly bind to the lymphocytes, and each lymphocyte can have a maximum of M RBC attached. Further assume that the initial concentration of lymphocytes, n_T, is sufficiently small compared to the initial concentration of RBC, x_0, that we can ignore the possibility of multiple lymphocytes binding to a single RBC. More precisely, we assume $Mn_T/x_0 \ll 1$ so that RBC are in excess, even when each lymphocyte is completely surrounded by RBC.

Let n_i be the concentration of lymphocytes with i RBC attached, $i = 0,1,\ldots,M$. Then according to the law of mass-action

$$\frac{dn_0}{dt} = -k_1 M x n_0 + k_{-1} n_1$$

$$\frac{dn_i}{dt} = k_i [M-(i-1)] x n_{i-1} - [k_{i+1}(M-i)x + k_{-i} i] n_i + k_{-(i+1)}(i+1) n_{i+1} , \quad i=1,\ldots,M-1 \qquad (8)$$

$$\frac{dn_M}{dt} = k_M x n_{M-1} - k_{-M} M n_M ,$$

where x is the concentration of unattached RBC, and k_i and k_{-i} are the rate constants describing the attachment and detachment, respectively, of the ith RBC to a lymphocyte. At $t = 0$, $x(0) = x_0$. Thus

$$x(t) = x_0 - \sum_{i=0}^{M} i n_i \quad . \tag{9}$$

One can derive either directly from the law of mass-action or from Eqs. (8) and (9) that

$$\frac{dx}{dt} = - \sum_{i=0}^{M-1} k_{i+1}(M-i)x n_i + \sum_{i=1}^{M} k_{-i} i n_i \quad . \tag{10}$$

If we assume that the binding of RBC to a lymphocyte is independent of the number of RBC already attached, then all the k_i, i=1,...,M, are equal. Similarly, if we assume that the rate of dissociation of a particular RBC from a lymphocyte is independent of the number of other RBC attached to the same cell, then all the k_{-i} are equal. Under the condition that

$$k_i = k_f \quad , \quad k_{-i} = k_r \quad , \quad i=1,\ldots,M \quad , \tag{11}$$

Eq. (10) becomes

$$\frac{dx}{dt} = - k_f \sum_{i=0}^{M-1} (M-i)x n_i + k_r(x_0 - x) \quad ,$$

which because of conservation of lymphocytes further simplifies to

$$\frac{dx}{dt} = - k_f M n_T x + (k_r + k_f x)(x_0 - x) \quad . \tag{12}$$

Solving Eq. (12) subject to $x(0) = x_0$ we find

$$\tilde{x}(\xi) = \frac{\gamma_1(1-\gamma_2) - \gamma_2(1-\gamma_1)e^{-(\gamma_1-\gamma_2)\xi}}{1 - \gamma_2 - (1-\gamma_1)e^{-(\gamma_1-\gamma_2)\xi}} \quad . \tag{13}$$

where

$$\tilde{x}(\xi) = x(\xi)/x_0 \quad , \tag{14a}$$

$$\xi = k_f x_0 t \quad , \tag{14b}$$

$$\gamma_{1,2} = \frac{-(\alpha+\kappa-1) \pm \sqrt{(\alpha+\kappa-1)^2+4\kappa}}{2} \quad , \tag{14c}$$

$$\alpha = M n_T / x_0 << 1 \quad , \tag{14d}$$

and

$$\kappa = k_r / k_f x_0 \quad . \tag{14e}$$

4.1 Irreversible Binding

When RBC bind to the lymphocytes irreversibly, $k_r = 0$ and hence $\kappa = 0$. In this case $\gamma_1 = 0$, $\gamma_2 = 1-\alpha$ and

$$\tilde{x}(\xi) = \frac{1 - \alpha}{1 - \alpha e^{-(1-\alpha)\xi}} \quad . \tag{15}$$

An explicit solution to Eq. (8) can be obtained in a straightforward manner when $k_r = 0$. With $\tilde{x}(\xi)$ known and given by Eq. (15), we change time scales in Eq. (8) so that

$$d\tau = \tilde{x}(\xi) d\xi \quad . \tag{16}$$

Equation (8) becomes a linear, constant, coefficient system of ordinary differential equations. Although one can determine the eigenvalues of the system and proceed in the standard manner, it is more efficient to note that Eq. (8) in the τ-time scale is identical to the forward Kolmogorov equation for a pure birth process with birth rate $(M-i)k_f x_0$. Using results in Feller [12] or Perelson and Macken [13] one finds

$$\tilde{n}_i(\tau) = \binom{M}{i}[1 - e^{-\tau}]^i [e^{-\tau}]^{M-i} \quad , \tag{17}$$

where $\tilde{n}_i(\tau) \equiv n_i(\tau)/n_T$.

Lastly, we determine the dependence of τ on ξ. To simplify the notation we henceforth only work with nondimensional time. Thus we drop the tilde on ξ. By integrating Eq. (16) we find

$$\tau = \ell n \left(\frac{\alpha e^{\alpha t} - e^{t}}{\alpha - 1} \right) - \alpha t \quad . \tag{18}$$

The substitution of Eq. (18) into Eq. (17) yields

$$\tilde{n}_i(t) = \binom{M}{i} \left[\frac{e^{(1-\alpha)t} - 1}{e^{(1-\alpha)t} - \alpha} \right]^i \left[\frac{1 - \alpha}{e^{(1-\alpha)t} - \alpha} \right]^{M-i} \quad . \tag{19}$$

4.2 Reversible Binding

When the binding to the lymphocyte is reversible we use a probabilistic technique developed in polymer chemistry. Let p(t) be the probability that a RBC is bound to a lymphocyte at time t. Thus

$$p(t) = \frac{x_0 - x(t)}{x_0} \quad . \tag{20}$$

In deriving Eq. (8) we have implicitly assumed that a lymphocyte has M sites to which a RBC can bind. Let $p_L(t)$ be the probability that a site is bound. Because $x_0 - x(t)$, the concentration of bound RBC, must equal the concentration of occupied sites,

$$p_L(t) = \frac{x_0 - x(t)}{M n_T} = \frac{p(t)}{\alpha} \quad . \tag{21}$$

Assuming $k_i = k_f$ and $k_{-i} = k_r$ for all i is equivalent to assuming that the binding of red cells to lymphocytes are independent events. Because of this assumption the probability of a lymphocyte with i bound RBC is given by the binomial distribution. Consequently,

$$\tilde{n}_i(t) = \binom{M}{i}[p_L(t)]^i[1 - p_L(t)]^{M-i} \qquad (22)$$

or

$$\tilde{n}_i(t) = \binom{M}{i}\left[\frac{1-\tilde{x}(t)}{\alpha}\right]^i\left[\frac{\tilde{x}(t)-(1-\alpha)}{\alpha}\right]^{M-i} , \qquad (23)$$

where $\tilde{x}(t)$ is given by Eq. (13).

As a check on Eq. (23), consider the case in which the reactions are irreversible. Then $\tilde{x}(t)$ is given by Eq. (15). Evaluation of $p_L(t)$ shows

$$p_L(t) = \frac{e^{(1-\alpha)t}-1}{e^{(1-\alpha)t}-\alpha} = 1 - e^{-\tau} ,$$

and hence the previous solution, Eq. (17) is obtained.

4.3 Equilibrium Solution

At long times an equilibrium distribution of rosette sizes will be obtained. Let $\bar{n}_i$ and $\bar{x}$ denote the equilibrium values of $\tilde{n}_i(t)$ and $\tilde{x}(t)$, respectively. From Eq. (23) we see

$$\bar{n}_i = \binom{M}{i}\left[\frac{1 - \bar{x}}{\alpha}\right]^i\left[\frac{\bar{x} + \alpha - 1}{\alpha}\right]^{M-i} , \qquad (24)$$

and from Eq. (13), assuming $Y_1 > Y_2$,

$$\bar{x} = Y_1 .$$

A more elucidating form for the equilibrium size distribution can be obtained by setting the time derivatives in Eq. (8) to zero and solving the resulting system of algebraic equations. Using this approach one can show that

$$\bar{n}_i = \binom{M}{i}(K\bar{x})^i\bar{n}_0 , \qquad (25)$$

where $K = 1/\kappa$ is a nondimensional equilibrium binding constant. Further, because

$$\sum_{i=0}^{M} \bar{n}_i = 1 \qquad (26)$$

one finds

$$\bar{n}_0 = (1 + K\bar{x})^{-M}$$

and hence

$$\bar{n}_i = \frac{\binom{M}{i}(K\bar{x})^i}{(1 + K\bar{x})^M} . \qquad (27)$$

To see that Eqs. (27) and (24) are equivalent, we note from Eq. (12) that $\bar{x}$ satisfies the equation

$$(\bar{x} + \alpha - 1)(K\bar{x} + 1) - \alpha = 0 ,$$

and hence

$$\frac{\bar{x} + \alpha - 1}{\alpha} = \frac{i}{1 + K\bar{x}} . \qquad (28)$$

Rearrangement of Eq. (28) shows

$$\frac{1 - \bar{x}}{\alpha} = \frac{K\bar{x}}{1 + K\bar{x}} . \qquad (29)$$

The substitution of Eqs. (28) and (29) into Eq. (24) demonstrates its equivalence with Eq. (27).

4.4 Experimental Test

If $\bar{x}$ and the equilibrium size distribution of rosettes were measured experimentally, then a plot of $\bar{n}_i/\bar{n}_{i-1}$ vs $1/i$ would provide useful information. From the theory, Eq. (27), we expect

$$\bar{n}_i/\bar{n}_{i-1} = [(M + 1)\frac{1}{i} - 1]K\bar{x} . \qquad (30)$$

Thus such a plot should yield a straight line with slope $(M + 1)K\bar{x}$ and y-intercept $-K\bar{x}$. Hence both M, the maximum number of RBC that can bind to a lymphocyte, and K, a constant related to the free energy of binding of a RBC to a lymphocyte, can be determined.

5. DISCUSSION

A set of idealized models have been developed that describe the formation of EA-rosettes, i.e., antibody mediated lymphocyte - RBC aggregates. The first, a stochastic model, was aimed at describing the equilibrium size distribution of rosettes in terms of p, the probability that a red blood cell and lymphocyte stick together. Our method of determining p is rather primitive. We simply assume that if there are at least N antibodies in the area δA over which the lymphocyte and red cell interact then the two cells adhere. This is clearly a great simplification, but one that allows us to make correlations with experimental data and yet avoid all the details of the Ig-Fc receptor interaction. Bell and colleagues [3-8] have considered the two cell

interaction in great detail. Their theories--which involve the density of Ig molecules on the red cell surface; the density of Fc receptors on the lymphocyte; the affinity or equilibrium binding constant of the Fc receptor for Ig; the mobility of both the Fc receptor and surface Ig, and the nonspecific van der Waals, electrostatic, and entropic forces between cells--can be used to calculate the free energy change upon cellular adhesion and hence, via a Boltzmann distribution, the probability that two cells adhere. It would be of interest to compare this more detailed approach with the one used here. In particular the present model does not allow for the possible accumulation of Fc receptors in contact areas and their depletion from exposed lymphocyte surfaces. This affect may hinder the formation of large rosettes.

Given that the probability that two cells adhere is known, we then used a binomial distribution to calculate the probability of a rosette of size m. Two assumptions were made here. First, that a maximum number, M, of RBC could simultaneously bind to a lymphocyte, and second that the bindir of RBC are independent events. By looking at rosettes under a microscope one gets the impression that at most 20 or 30 RBC bind to a lymphocyte. However, because RBC are readily deformable, the maximum number that are actually observed in an experiment may depend on the experimental conditions. Thus M should be viewed as an adjustable parameter that is roughly 20 or 30 but whose value may be somewhat dependent on the validity of our second assumption--the independence of successive red cell binding events. When conditions are such that only a few RBC bind per lymphocyte, one would expect that the independence assumption is rather good. However, as the number of RBC binding to a lymphocyte increases, one would expect steric effects to become important; cells may have to deform to fit into available gaps or wait until the gaps enlarge due to the Brownian movement of the Fc receptors within the plane of the lymphocyte membrane. Thus, we expect that under conditions in which large rosettes are formed more refined theories may be necessary.

Preliminary exploration of the ability of this probabilistic model to account for the data of Beckett et al. [11] indicated that some dispersion in the value of N was needed. Because of biological variability, it is not unexpected to find that different lymphocytes might require different numbers of chemical bonds to mediate red cell adhesion. The truncated Poisson which we used, with $N_{min} = 2$ and $\mu = 4$, suggests that the variability is limited and that rather few bonds, less than 10, are required to mediate adhesion. Analysis of further experimental data will be required to confirm this result.

The second model that we developed, the kinetic model, is more in conformity with the theme of this section of the conference, nonlinear dynamics. Under the assumption that the lymphocyte population can be treated as homogeneous and the binding of a red cell to a lymphocyte codified in terms of a forward and reverse rate constant, a set of nonlinear ordinary differential equations describing the growth of rosettes was formulated. Under the assumption of independence of red cell binding, these nonlinear equations were solved analytically, both for the dynamic and equilibrium behavior of the aggregation process.

Although we have centered our discussion around rosette formation, it should be clear that the methods we have developed are applicable to many other examples: adhesion between cells of various types, between lipid vesicles, and between particles such as latex beads; all of which have become of growing interest [cf. 15-21]. From a mathematical viewpoint, our analysis of the kinetic model provides an explicit solution to a problem studied by Gani [22-23] involving the attachment and detachment of antibodies to a virus or bacteria. Within the context of Gani's work, Eqs. (15) and (19) for the kinetics of irreversible binding have been derived by other means.[23-24]

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